American Journal of Hematology 59:42–45 (1998)

Food-Bound B12 Absorption and Serum Total Homocysteine in Patients With Low Serum B12 Levels

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This study was undertaken to determine whether measurements of serum total homocysteine (Hcys) and bound B12 absorption are useful in determining which patients with low- or low-normal levels of serum B12 are B12 deficient. In 40 patients with low or borderline serum levels of B12, food-bound B12 absorptions were determined using a body counter in an iron room, and were related to serum total Hcys levels. Food-bound B12 absorption was decreased in 16 patients and in an additional four, absorption of the free vitamin was also decreased. Homocysteine levels were elevated in four of the 16; in three of the four who had both decreased bound and free B12 absorptions, Hcys was elevated. If elevation of the Hcys level indicates tissue deficiency of B12, the 75% incidence of normal levels of Hcys in these patients with low food-bound B12 absorptions suggests the existence of a cohort of patients who may be at risk to develop, but have not yet developed, B12 deficiency. Only long term follow-up will reveal how many ultimately will become B12 deficient. Am. J. Hematol. 59:42–45, 1998.

Key words: homocysteine; serum B12; B12 deficiency; food-bound B12 absorption; free B12 absorption

INTRODUCTION

Recent studies have reemphasized the difficulties inherent in using the blood level of vitamin B12 as the criterion of B12 deficiency [1–3]. Various tests have been proposed to improve diagnostic specificity and sensitivity. These include the standard hematological and neurological criteria of B12 deficiency, measurements of biochemical indices of vitamin B12 deficiency, namely blood levels of methylmalonic acid and total homocysteine (Hcys) [1,4–7], and lastly, establishment of a defect in absorption, preferably using bound rather than free vitamin [8,9]. The present study relates the absorption of food-bound B12 as measured by body counting to serum levels of total Hcys in patients with low or borderline (170–220 pg/ml) levels of serum B12.

PATIENTS AND METHODS

Subjects

B12 absorptions were measured in 40 patients in response to requests from the physicians caring for the patients at our hospital and from satellite VA installations. Clinical reasons for their requests included a low or marginally normal serum B12 level in association with anemia with or without macrocytosis, macrocytosis without anemia, and/or a variety of neuropsychiatric problems. No patient had had gastrointestinal surgery or clinical malabsorption. None of these patients were described in previous studies. The patient population was largely male (38 of 40) with a median age of 70 and a range of 42 to 90. Patients were fasted from midnight and blood was drawn in the morning for routine hematological study, serum B12/folate, gastrin, and Hcys. Serum was removed rapidly from cells and stored at −20°C until the time of assay. Egg yolk-bound B12 absorption was then measured.

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Complete blood work, including red cell indices, was performed using a Coulter (Hialeah, FL) Model S-Plus II

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Received for publication 8 December 1997; Accepted 13 May 1998

counter. Peripheral blood for red cell morphology and neutrophile segmentation was available on some patients.

Serum B12 and Folate

Serum B12 and folate were measured using a commercial radioassay kit (Becton-Dickinson, Orangeburg, NY) in which both vitamins were measured simultaneously. Normal B12 levels ranged from 220–940 pg/ml with pernicious anemia patients less than 170 pg/ml. The range of 170–220 was considered borderline. The normal range for serum folate was 3–14 ng/ml.

Serum Gastrin

Levels of serum gastrin were determined using a commercial radioimmunoassay kit (Becton-Dickinson). The normal range was 0–200 pg/ml.

Serum Hcys

Serum Hcys was determined as described previously [10]. Median ± SD for healthy subjects was 9.1 ± 1.5 μmol/l with a range of 6.9–12.1 μmol/l. These values are close to published figures for a similar method [11] and for that of Stabler et al. [5]. In evaluation of results, any value greater than three SD from the mean, i.e., 13.6 μmol/l, was considered abnormal.

Food-bound B12 Absorption

The preparation of an omelette from a capsule of ^{57}Co B12 and dessicated egg yolk, its administration and measurement of the amount of the ^{57}Co B12 absorbed by the body in the iron room counter was previously described [12]. Free ^{57}Co B12 absorption was also determined when food-bound B12 absorption was subnormal.

RESULTS

The absorptions of bound and free B12 and the serum Hcys levels for 40 patients are given in Table I with the hematological and serum B12 and folate levels in Table II.

Of the four patients in Group I (with decreased bound and free B12 absorption), three had increased Hcys levels (greater than 13.6 μmol/l), macrocytic anemia, serum B12 levels less than 100 pg/ml, elevated serum gastrin levels, and hematological responses to B12 therapy.

There were 12 patients (Group II) with decreased food-bound B12 but normal absorption of free B12. Only one had an elevated Hcys level, a patient with myelodysplastic syndrome who manifested no hematological response to B12 therapy. As a group, a moderate to no anemia was present (Table II), with macrocytosis found in one third. Six of the 12 had elevated serum gastrin levels. In one patient, restudied five years later, the absorption of free B12 had decreased from 78% to a low normal level of 37%. There was no significant change in the 5–6% absorption of food-bound B12.

Of the 24 patients with normal food-bound B12 absorptions (Group III) all but three had normal levels of Hcys. Of the three, two had borderline levels of Hcys (14.3 and 14.4 μmol/l) and the third, with a level of 18.3 μmol/l, had a normal level of serum folate with neither anemia nor macrocytosis. As a group, these patients had a higher level of serum B12 and a lower level of serum folate than Group II. Serum gastrin levels were elevated modestly in two of the group.

TABLE I. Food-bound and Free B12 Absorption and Serum Homocysteine in 40 Patients With Low- or Low-Normal Serum Vitamin B12 Levels*

		B12 absorption (%)		
	n	Food-bound	Free	Homocysteine (μmol/1)
Group I	4	0.2–9.0[a]	2–21	5.6–50.7
		1.4[b]	8.6	18.1
Group II	12	0.7–10.3	34–89	6.0–18.7
		5.6	56.5	9.0
Group III	24	12.8–43.0	—	4.6–18.3
		20.0		9.2
Controls	15	12–39		
		25		
	31		33–95	
			66	
	18			6.1–12.1
				9.1

*Group I, low food-bound and free B12 absorption; Group II, low food-bound and normal free B12 absorption; Group III, normal food-bound B12 absorption.
[a]Range.
[b]Median.

TABLE II. Serum B12, Folate, MCV, and Hb in 40 Patients With Low- or Low-Normal Serum B12 Levels*

	n	Serum B12 (pg/ml)	Folate (ng/ml)	MCV (>94 fl)	Hb (g/dl)
Group I	4	32–114[a]	6.6–13.6	3	10.3–15.0
		37[b]	10.7		11.8
Group II	12	15–216	3.0–24.0	4	9.6–14.5
		119	9.8		13.3
Group III	24	32–265	4.1–19.3	8	9.3–15.7
		137	7.4		13.7

*Hb, hemoglobin; MCV, mean corpuscular volume; Group I, low food-bound and free B12 absorption; Group II, low food-bound and normal free B12 absorption; Group III, normal food-bound B12 absorption.
[a]Range.
[b]Median.

DISCUSSION

Absorption of food-bound B12 was decreased in 16 of 40 patients with low or borderline levels of serum vitamin B12. In four of the 16, absorption of the free vitamin

was decreased, a finding presumably explained by intrinsic factor deficiency. Hcys levels were increased in three of the four, all of whom had hematological evidence of B12 deficiency; the one patient with normal Hcys lacked such blood findings.

Twelve patients with decreased absorption of bound vitamin B12 absorbed the free vitamin normally. Hematological evidence of B12 deficiency was absent or equivocal and serum Hcys levels were normal in all but one patient with myelodysplastic syndrome. It should be noted that, had it been assayed, an elevated level of methylmalonic acid might have been found in some additional patients [7,13]. The findings of normal Hcys levels in patients with a decrease in bound B12 absorption suggest that at least some are only in the early stage of vitamin B12 deficiency; that is, where cell B12 stores are decreased, serum B12 levels are low or marginal, and biochemical/hematological indices of B12 deficiency are absent [14,15]. The rate of progression from this early stage of deficiency to one manifested by elevated Hcys is slow and in some cases may never occur [15]. When seen, it is probably usually dependent on a decline in intrinsic factor production as noted in one of our patients and as described by Carmel [16], but some patients with a documented lack of intrinsic factor may remain in the early stage of B12 deficiency for years without treatment [17]. The reported cases of B12 responsive megaloblastic anemia with normal absorption of free vitamin [18–20] indicate, however, that some patients may manifest B12 deficiency despite an amount of intrinsic factor adequate to maintain normal free B12 absorption. Given that our patients who do not absorb food-bound but do absorb free B12 usually lack sufficient pepsin/HCl to release the bound B12 [21], only long-term follow-up will reveal how many develop B12 deficiency for that reason alone, how many will require the additional defect of depressed levels of intrinsic factor, and finally how many will never become B12 deficient.

More than half the patients (25) in this study had normal absorptions of food-bound B12, a finding similar to that of a previous study [12]. Hcys levels were normal in 12 of these patients. Some [22] but not all [23] studies have suggested that low dietary B12 may be a cause of low serum B12 levels. The Hcys data is against tissue deficiency in our patients but, given the insensitivity of Hcys for early stages of B12 deficiency, a dietary factor cannot be excluded. The explanation for the low serum B12 in these patients remains uncertain. A recent study [24] found a decrease in transcobalamin I in about one fifth of patients with low levels of serum B12 and normal absorption of bound B12.

It is difficult to compare our data with those of other studies, all of which used the Schilling-type measurement of urine radioactivity in which the amount of radioactivity is so small as to make it difficult to separate controls from B12 malabsorbers [21]. This was particularly true when chicken serum was used as the binder [25]. Comparisons are further complicated because preclinical B12 deficiency may be present for months or years and patient populations therefore differ widely in the duration of disease.

The preferred diagnostic approach to the patient with low serum B12 and equivocal or no neurological or hematological evidence of B12 deficiency is uncertain. The scope of the problem has been expanded by the finding of increased levels of methylmalonic acid and Hcys in nonhospitalized elderly individuals with serum B12 levels in the 200–350 pg/ml range [26–29]. Should all such patients have metabolite levels assessed [28]? Our bound B12 absorption and Hcys data would suggest that better than half of such patients may not be B12 deficient. Furthermore, even when bound B12 absorption was decreased, Hcys levels were normal in patients with equivocal clinical and hematological abnormalities. These findings suggest that routine metabolite assays may be largely unrewarding. This is not a trivial question given the cost of assays and the number of patients involved.

Testing for a defect in bound B12 absorption might help separate patients at risk for B12 deficiency from those who are not. However, as discussed previously, the Schilling procedure suffers from poor separation of controls from abnormals, and a need for a 24-hour urine collection makes it difficult in the elderly, whereas our method, free of the above problem, requires a shielded site to reduce natural background counts. Furthermore, neither of these procedures is inexpensive.

Initially, elevated serum gastrins were found in a small group of patients with a selective decrease in food-bound B12 absorption [30]. However, in subsequent studies [12] as well as the present one, elevations of serum gastrin levels have been observed less frequently (in about half the patients). Other workers have noted an even poorer correlation [9].

In summary, the diagnostic approach to the patient with a low serum B12 level in the absence of hematological or neurological evidence of B12 deficiency, remains problematic.

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